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Reconstruction of the forehead acoustic properties in an Indo-Pacific humpback dolphin (*Sousa chinensis*), with investigation on the responses of soft tissue sound velocity to temperature

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Abstract

Computed tomography (CT) imaging and ultrasound experimental measurements were combined to reconstruct the acoustic properties (density, velocity and impedance) of the head from a deceased Indo-Pacific humpback dolphin (*Sousa chinensis*). We extracted 42 soft forehead tissue samples to estimate the sound velocity and density properties at room temperature 25°C. Hounsfield Units (HU) of the samples were read from CT scans. Linear relationships between the tissues' HUs and velocity, and HUs and density were revealed through regression analyses. The distributions of the head acoustic properties at axial, coronal and sagittal cross sections were reconstructed, suggesting that the forehead soft tissues were characterized by low-velocity in the melon, high-velocity in the muscle and connective tissues. Further, the sound velocities of melon, muscle and connective tissue pieces were measured under different temperatures to investigate tissues' velocity responses to temperature. The results demonstrated nonlinear relationships between sound velocities and temperature. This study represents a first attempt to provide general information on acoustic properties of this species. The results could provide meaningful information for understanding the species' bioacoustic characteristics and for further investigation on sound beam formation of the dolphin.

Key Words: Indo-Pacific humpback dolphin; acoustic property; CT scan; nonlinear.

PACS Number(s):43.80.Ka

I. INTRODUCTION

The Indo-Pacific humpback dolphin (*Sousa chinensis*) has several separate populations in Chinese coastal waters.[1, 2] Previous studies on the species have revealed its vocalization and audiogram properties.[3-7] Similar to other delphinid species, the Indo-Pacific humpback dolphin produces high frequency echolocation clicks for navigation and prey detection, terminal buzz echolocation clicks during foraging behavior and whistles for communication.[3-12] However, these sounds do not always correspond to one specific behavior. Clicks can also occur during socializing and whistles sometimes appear during foraging, indicating the complexity of sound generation and acoustic behavior in this species.[7] The propagation of the dolphin sounds in the marine environment is affected by the physical properties of water and the variation in environmental parameters e.g. salinity, depth, bottom substrate and temperature.[8] The ability of marine mammals to echolocate may also be affected by the noise of similar frequencies from anthropogenic activities e.g. marine construction (pile driving and drilling), seismic surveys, military sonar, underwater blasting operations, fishing and vessel traffic.[8, 13-18]These anthropogenic activities were correlated with physical injury and in some cases caused mortality of dolphins.[15, 16, 19-21]Previous research has shown that boat traffic could influence the acoustic and surface behavior of Indo-Pacific humpback dolphins.[13] The combined and cumulative effects from anthropogenic threats have caused concern for the conservation status of Indo-Pacific humpback dolphins.[22, 23, 24]

Studies have been conducted on Indo-Pacific humpback dolphins, [4-7, 25] but only a few studies have been conducted on its biosonar sound production and reception systems of the species.[4, 25] In comparison, studies have been conducted on sound emission, propagation and beam formation in other odontocete species by combining CT scanning, physical measurements and numerical simulation.[11, 12]

In recent years, CT scanning has been used extensively in studies on odontocetes.[11, 12, 24-31] It allows

63 researchers to study the inner structures of dolphins in high resolution without dissections and thus improve
64 efficiency in data analysis.[11, 29] Cranford *et al* combined CT and dissections to investigate the forehead
65 sound production system of many odontocete species and proposed a universal sound generation
66 mechanism for odontocetes.[11] CT scanning results were expressed in CT scanning numbers, called
67 Hounsfield Unit (HU), which has been found linearly related to sound velocity and density of soft tissues
68 from a Cuvier's beaked whale (*Ziphius cavirostris*),[26] a young individual of the Yangtze finless porpoise
69 (*Neophocaena asiaeorientalis asiaeorientalis*)[27] and a pygmy sperm whale (*Kogia breviceps*)[30]. To date,
70 sound velocity data of the forehead tissue are also accessible in other species, North Atlantic Pilot whale
71 (*Globicephala melaena melaena*),[32] sperm whale (*Physeter catadon*),[33] Chinese river dolphin (*Lipotes*
72 *vexillifer*),[34, 35] and dwarf sperm whale.[36] These studies include concrete descriptions of the anatomical
73 structures of the odontocete head. Numerical head models of odontocetes were established based on
74 anatomical analysis through CT scanning study, which further facilitated the investigation on acoustic field
75 and the beam formation process on these species.[12, 37-40]

76 Previous measurements of the tissues in odontocetes were derived from experiments conducted at
77 room temperature range (20-25℃), which likely made them less representative of tissues from odontocetes
78 *in vivo*.[26, 27, 30] The effects of temperature on soft tissues' sound velocity have been revealed in Cuvier's
79 beaked whale (*Z. cavirostris*),[26] dwarf sperm whale (*Kogia sima*)[36] and sperm whale.[41] Part of these studies
80 showed linear relationships between temperature and sound velocity of tissues (forehead blubber,
81 mandibular blubber, forehead acoustic fat, mandibular acoustic fat, muscle, connective tissue). In most
82 cases, the sound velocity of soft tissues howed nonlinear changes with temperature.[36, 41, 42] In this study, we
83 also address the temperature issue by using CT imaging and ultrasound experimental measurements of
84 acoustic tissues. More importantly, this study provides information on the acoustic properties of forehead

tissue structures in the Indo-Pacific humpback dolphin. The results will provide basic anatomical and physical properties which are important for further sound production and propagation studies of the species.

II. MATERIALS AND METHODS

A. CT scanning

A dead female Indo-Pacific humpback dolphin was found stranded in Quanzhou waters on 14 March, 2015. The specimen was retrieved and then kept frozen in a -25°C chamber until further investigation. The specimen was thawed completely before taken to CT scanning. A CT scan of the head, morphological measurements, and dissection of the body were performed on 23 May 2015. The body length was 2.7 m, weight 280 kg, and the age was unknown. Given its length, weight, and pink coloration, this specimen was considered to be an adult. [43] CT scanning of the dolphin's head was carried out at the Radiology Department of Affiliated Zhongshan Hospital of Xiamen University. A GE Light Speed VCT 64 Slice CT scanner (*GE, Pittsburgh, Pennsylvania, USA*) was used for scanning with a slice width of 0.625mm. All scanned images were collected with a 120kV×600 mA power setting with a matrix size of 512 x 512, and saved in DICOM format. The scanned images were processed using the software Mimics 16.0 (*Materialise, Leuven, Belgium*) to read HU values of the different head tissues. HU describes the CT numbers derived by comparing the linear attenuation coefficient of a voxel with that of water.[44] Water at room temperature was used as reference to obtain the HU values of air and hard bone, -1000 and >1000, respectively. For mammalian soft tissues, the HU range is between –100 and 100, with fatty tissues at the low end and denser connective tissues at the higher end.[42] Using CT imaging, the internal structures, namely melon, skull structures, teeth and air components of the dolphin’s head were reconstructed in three dimensions as shown in Fig. 1a. The respective positions of the melon, blubber, mandibular fat, muscle and connective tissues were determined by analyzing the CT images.[11, 26, 27, 30]

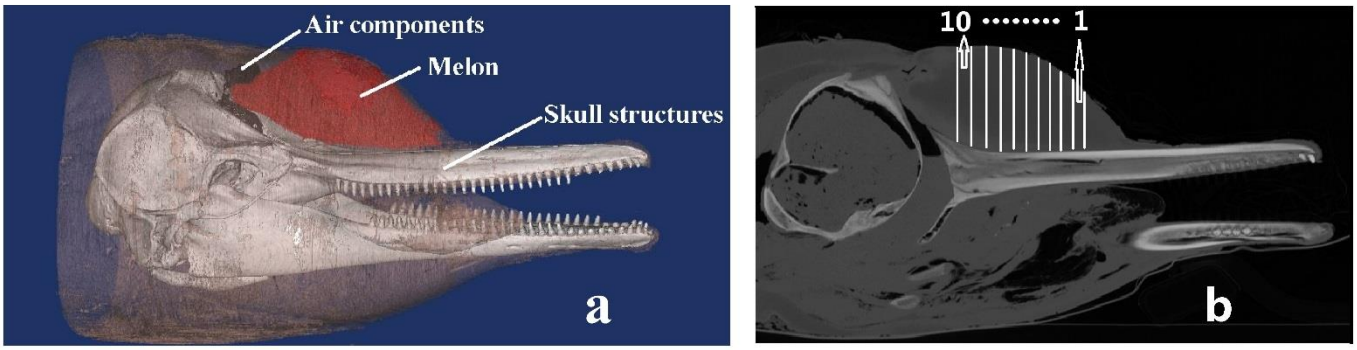

Fig. 1 (Color online) (a) A reconstructed head of an Indo-Pacific humpback dolphin (*Sousa chinensis*) specimen from CT imaging in three dimensions, where Air components, Melon and Skull structures could be observed clearly. (b) A CT image showing the sagittal cross section of an Indo-Pacific humpback dolphin's head (lateral view). The Arabic numerals indicate the transverse slices.

B. Sound velocity measurement

After the specimen was completely thawed and scanned, the sound velocity of the dolphin's forehead tissues was measured using an ultrasound velocimeter at room temperature (25°C). The velocimeter measures the sound velocity of a tissue by estimating the time difference between the different reflected waves and the thickness of the measured sample, following Soldevilla *et al*,[26] Wei *et al*[27] and Song *et al.*[30] We applied the same sound velocity measurement system as used by Song *et al*[30] except that the experiments were conducted in water. In this paper, the frozen and thawed dolphin forehead soft tissues were sectioned into ten transverse slices along the body axis (see Fig.1b and 2a). Each transverse slice was further cut into several smaller pieces, e.g. slice 7 was cut into 7 pieces, as shown in Fig. 2b.

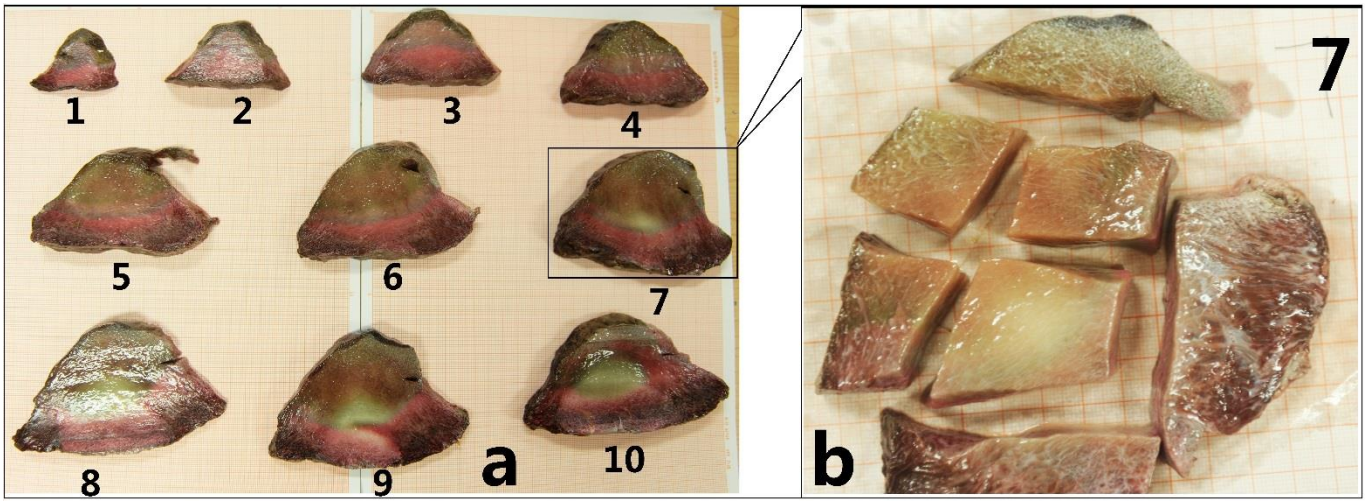

Fig. 2 (Color online) (a) Head tissue slices (1-10) (see Fig. 1b) from an Indo-Pacific humpback dolphin (*Sousa chinensis)* used for CT scanning to determine acoustic properties. (b) The slices were further cut into smaller pieces to facilitate the analyses, here, showing the individual pieces from slice 7.

Tissues were divided into three categories: melon, muscle and connective tissue according to the tissues' location, texture and color.[11, 26, 27, 30] Sound velocity measurement of a tissue sample was rejected if: (1) its size was smaller than the cylinder probe (1.5 cm in diameter) of the velocimeter; (2) its shape or size was greatly uneven; (3) it was potentially a mixture of two different tissues based on tissue location, color and texture. These procedures were conducted to ensure homogeneity of the analyzed samples. In total, 56 tissue samples were obtained, of which 42 samples (22 melon, 7 muscle and 13 connective tissue, shown in Fig. 3a) satisfied the above three criteria and were chosen for measurements. To measure the tissue samples sound velocities the same oscilloscope (*TDS 1012C-SC, Beaverton, Oregon*) and Olympus 5073PR (*Waltham, Massachusetts, USA*) ultrasonic pulser-receiver were used as described by Song *et al*.[30]

In addition, the potential effects of temperature on the acoustic properties of the soft tissues were investigated by using water baths at temperatures ranging from 20.8°C to 39.0°C. The measurements were conducted using the same measuring system as in room temperature. During the water bath experiments, temperature was the only variable that was changed, recorded and displayed in real-time by a

138 microcomputer. The time difference (Δt) between first reflection wave (from the sample's upper surface)
139 and second reflection waves (from the sample's lower surface) was measured with an oscilloscope.
140 Thickness of the samples were considered unchanged.

141 For each slice, there could be melon, muscle and connective tissue in it. Taking slice 7 as example,
142 shown in Fig.2, we cut the slice was into seven pieces and these pieces were composed of melon pieces,
143 muscle pieces and connective tissue pieces. For all pieces, which were cut from ten transverse slices, we
144 randomly selected three slices and picked up one melon piece from each slice. In consequence, three melon
145 pieces from three slices were chosen to investigate the temperature issue. The same procedure was
146 presented on muscle and connective tissues. Thus, there were totally nine pieces of soft tissue samples used
147 to study the effect of temperature on tissue velocity. The sound velocities of the three samples from each
148 tissue were computed to obtain a mean value to represent the tissue's sound velocity under each temperature.
149 Polynomial regression analysis (using all available samples) were used to investigate the relationships
150 between sound velocity and temperature. After this, we split the whole temperature range 20-39°C into 20-
151 32°C and 32-39°C. Linear regression analysis between velocity and temperature under these two
152 temperature ranges were conducted respectively.

153 After obtaining the polynomial relationships between sound velocity and temperature in melon,
154 muscle and connective tissue, we combined the data with the measured sound velocity distribution derived
155 from CT scanning under the room temperature (25℃), to reconstruct the sound velocity distributions at
156 temperature 37℃, assuming that HU values remained constant for the two temperatures (25°C and 37°C).

157 **C. Density measurement**

158 The density measurement system was referred from a previous study by Song *et al*[30]. The samples
159 were measured by an electronic balance with an accuracy of 0.001 g to obtain the mass. Each sample was

160 measured three times to calculate the mean $\bar{m}$. For sample volume measurement, water was firstly added
161 into a cylinder with the accuracy of 1 ml to obtain an original water volume V_1. In the following step, the
162 sample piece was immersed into the cylinder and this would create a new volume V_2. The volume
163 difference ΔV ($V_2 - V_1$) equaled the sample volume. This process was repeated three times and each
164 sample had an average volume differences as $\Delta\bar{V}$. The density could be computed by following formula:

165 $$\rho = \bar{m} / \Delta\bar{V} \quad (1)$$

166 In measuring the volumes, muscle and connective samples were denser than water, but melon samples were
167 lighter than water and could float on the water surface. In this case, a glass rod was used to press the sample
168 under water and the immersed volume of the rod was deducted from ΔV to acquire the value representing
169 the sample volume.

170 ## III. RESULTS

171 The results of the CT scanning, expressed in Hounsfield Units (HU) and the sound velocity
172 measurements showed that the soft tissues' HU were linearly related to the tissues acoustic characteristics
173 (sound velocity, density and acoustic impedance), as shown in Fig. 3. There was a significant linear
174 correlation (df = 40, $p<0.001$, $r^2=0.91$) between sound velocity (c) and HU (Fig. 3a), given by the equation:

175 $$c = 1.67HU + 1479.33 \text{ (m/s)} \quad (2)$$

176 There was also a significant linear correlation (df = 40, $p<0.001$, $r^2=0.81$) between density (ρ) and HU
177 (Fig. 3b), given by the equation:

178 $$\rho = 0.50HU + 980.09 \text{ (kg/m}^3\text{)} \quad (3)$$

179 Finally, the acoustic impedance $\boldsymbol{Z_s}$ had a linear relationship (df = 40, $p<0.001$, $r^2=0.92$), given by the
180 equation:

181 $$Z_s = 2.40HU + 1456.16 \text{ (}10^3\text{ Pa·s/m)} \quad (4)$$

The linear relationships were then combined with the HU distributions shown in Fig. 4a to reconstruct the detailed distributions of sound velocity, density, and acoustic impedance, at three different head cross sections (axial, coronal, and sagittal) as shown in Figs. 4b–4d.

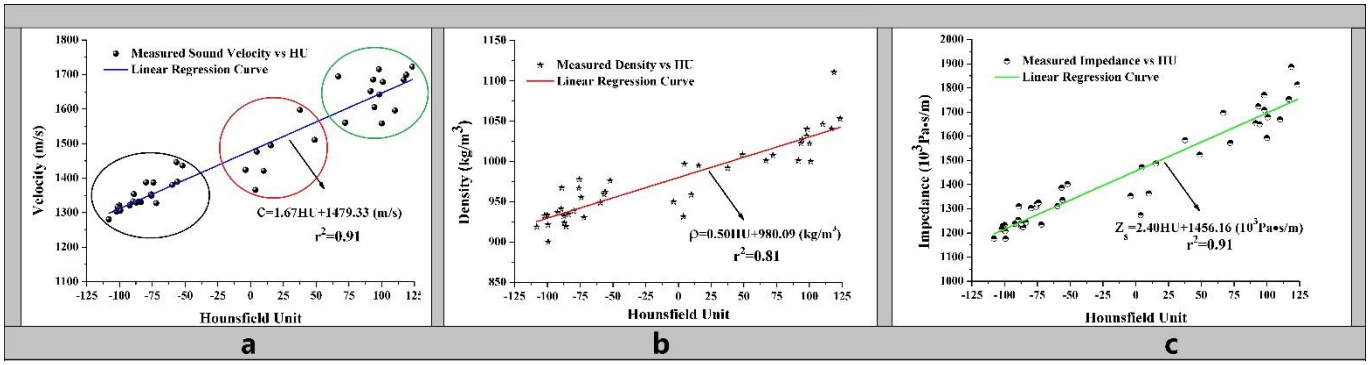

Fig. 3 (Color online) The regression line shows the linear relationships between (a) sound velocity vs Hounsfield Unit (HU), (b) density vs HU, and (c) impedance vs HU, based on analyses of 42 Indo-Pacific humpback dolphin (*Sousa chinensis*) forehead soft tissue samples. The scatters within the black, red and green ellipses in (a) represented melon pieces, muscle pieces and connective tissue pieces respectively.

The air filled regions, the skull structures and the soft tissues are all clearly identified in Fig. 4. The forehead soft tissues could further be separated into three tissue types; melon, muscle, and connective tissues, as indicated by different colors, with melon in blue, muscle in green and dense connective tissue in yellow (Fig. 4). The nasal passage can be identified between the rostrum and cranium and the symmetry of the left and right passages found in the present work is much higher than that found in e.g the pygmy sperm whale.[30] Two air sacs were identified posterior and dorsal of the melon and a third air sac was identified by the premaxilla. For skull structures, the HU values could be read from CT scans. We combined the linear relationships with the HU values of the skull to obtain the sound velocity and density distributions of the skull structures. In Fig. 4, the forehead skull structures (the cranium, rostrum and mandible) returned the highest density, sound velocity and acoustic impedance measurements. The results revealed that the melon was encased by muscle tissue that possessed a higher HU, sound velocity, density, and acoustic impedance

than the melon. Further, dense connective tissue was found to wrap the muscle peripherally in the area tangent to the nasal passage and rostrum. These indicated that the placement of the soft tissues, skull structure and air regions follow a clear organization.

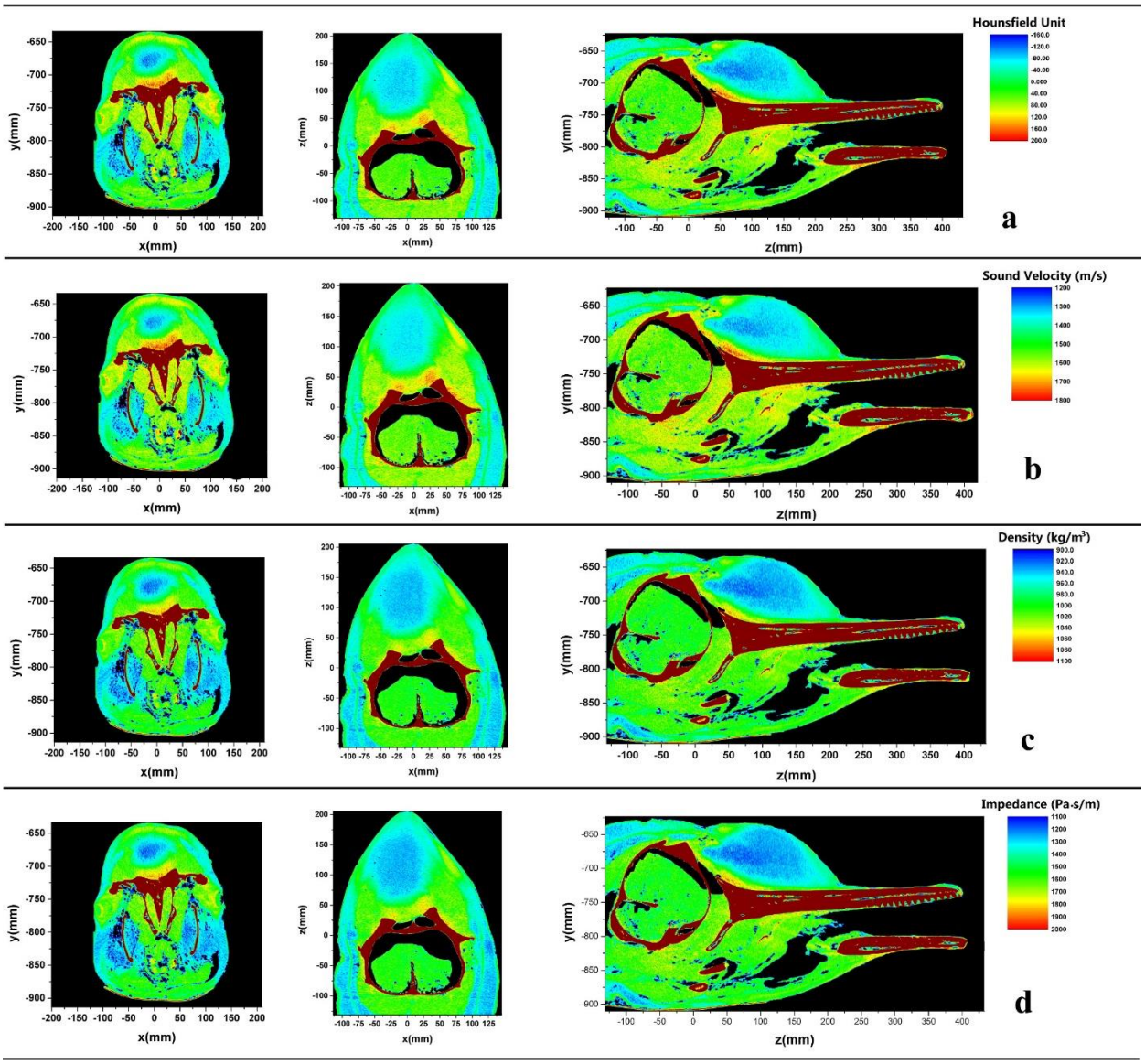

Fig. 4 (Color online) Distributions of head tissues measurements from an Indo-Pacific humpback dolphin (*Sousa chinensis)* for: (a) HU, (b) velocity, (c) density, and (d) acoustic impedance. The left image shows the coronal cross section, the middle image shows the axis cross section, and the right image shows the sagittal cross section. The colored legends show the value ranges of the properties (HU, sound velocity, density, and impedance).

The results showed that the tissues' sound velocities had a significant nonlinear inverse relationship

with temperature (Table I, Figs. 5a-c). Therefore, a nonlinear polynomial regression analysis was applied to investigate the relationships between sound velocity and temperature, shown in Fig. 5 and Table. I. The results in Fig. 5 demonstrated that the sound velocity was highest in the connective tissue at all temperatures followed by muscle and melon. The numerical values suggest that melon and muscle tissues have steeper slopes than connective tissue in the studied temperature range, which indicates that the sound velocity in the connective tissue is less affected by temperature change.

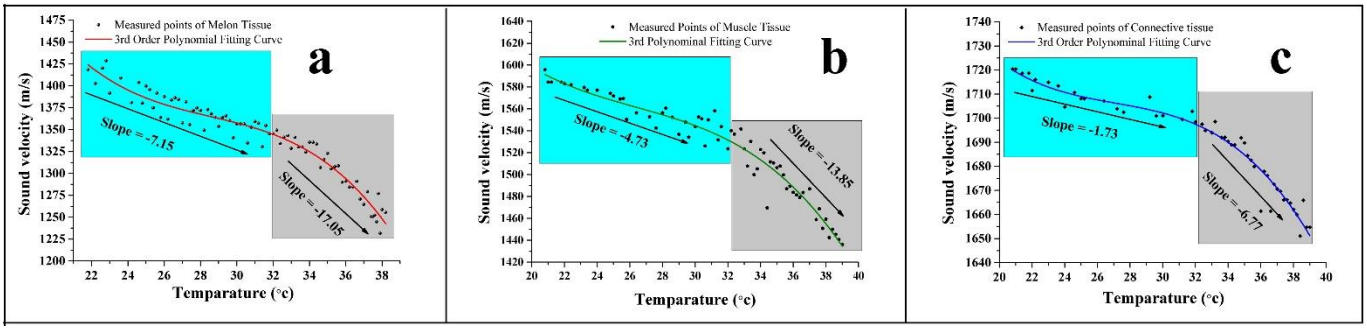

Fig. 5 (Color online) The response of soft tissues' sound velocity to temperature as described by non-linear regression: (a) melon tissue, (b) muscle tissue and (c) connective tissue sound. The slopes were derived by fitting linear regressions to data within the temperature ranges 20°C -32°C and 32°C -39°C, respectively.

Table. I Non-linear relationships between the Indo-Pacific humpback dolphin (*Sousa chinensis)* forehead soft tissues' sound velocity and temperature. B1 is the polynomial equation's monomial coefficient, B2 the quadratic coefficient, and B3 the cubic coefficient. Pearson coefficients (r^2) indicated the observed data are explained well by the non-linear regressions.

Tissue type	B1	B2	B3	Intercept	r^2	p value
Melon	-211.422	7.227	-0.084	3471.999	0.948	< 0.001
Muscle	-82.292	2.956	-0.037	2359.943	0.949	< 0.001
Connective tissue	-54.918	1.979	-0.024	2224.844	0.955	< 0.001

Clearly, the polynomial regression analysis revealed a nonlinear relationship between tissues' velocity and temperature. And in Fig.5, it could be observed that the sound velocity decreased at a faster rate in high

temperature range for all the tissues. Therefore, the temperature range was split into two parts, 20-32°C and 32-39°C. For range 20-32°C, we applied linear regression to obtain its slope to describe the descending rate of velocity as temperature increased. For range 32-39°C, linear regression was also conducted to obtain the slope. The results in Fig. 5 revealed sound velocity decreased in a faster rate above 32°C for melon, muscle and connective tissue. This further indicated a nonlinear behavior for tissues' velocity when temperature changed.

We used the three nonlinear equations above to compute the theoretical sound velocities for melon, muscle and connective tissue under temperature 25°C and compared these data to that derived from the experimental sound velocities (Table II). The sound velocity of melon from experimental data were averaged from the measured velocities of 22 melon sample pieces. The sound velocities of 7 muscle samples and 13 connective tissue samples were also averaged to get the corresponding average values to represent the sound velocity at temperature 25°C. The percentage errors for the all tissues between the computed and experimental results were considered acceptable, 2.89% for melon, 2.86% for muscle and 2.07% for connective tissue, respectively.

Table. II Results of sound velocity comparison between computed and experimental results for the three head tissue types, melon, muscle and connective tissue, from an Indo-Pacific humpback dolphin (*Sousa chinensis).*

Tissue type	Computing results (m/s)	Experimental results (m/s)	Error (%)
Melon	1390.824	1350.623 (n=23)	2.89
Muscle	1572.018	1527.1 (n=7)	2.86
Connective tissue	1713.769	1678.279 (n=11)	2.07

By using the numerical equations in Table. I and error values in Table. II, we attempted to reconstruct all 42 samples' sound velocities at temperature 37°C to illustrate the sound velocities for the dolphin *in vivo*. The temperatures 37°C and 25°C were used in the polynomial equations to estimate the sound velocity

difference (△c m/s) between the two temperatures, for melon (△c 102.528 m/s), muscle (△c 84.276 m/s) and connective tissue (△c 27.312 m/s). Assuming that these estimates were representative, the differences could then be applied to obtain the sound velocities at temperature 37°C for all 42 samples by subtracting 102.528 m/s from the 22 experimental melon samples' sound velocities, which were acquired at temperature 25°C, 84.276m/s from the 7 experimental muscle samples and 27.312 m/s from all 13 experimental connective tissues, respectively. In this way, the sound velocities of 42 samples at temperature 37°C were estimated. And the linear regression method was presented again to analyze the relationship between the newly obtained sound velocities and samples' HUs. A new linear relationship ($\boldsymbol{c} = 1.99HU + 1401.44$ (m/s), df = 40, $p<0.001$, $r^2=0.90$) between the estimated soft tissue's sound velocity at 37°C and HU were constructed and used to reconstruct the whole Indo-Pacific humpback dolphin head's sound velocity distribution at this temperature as shown in Fig. 6.

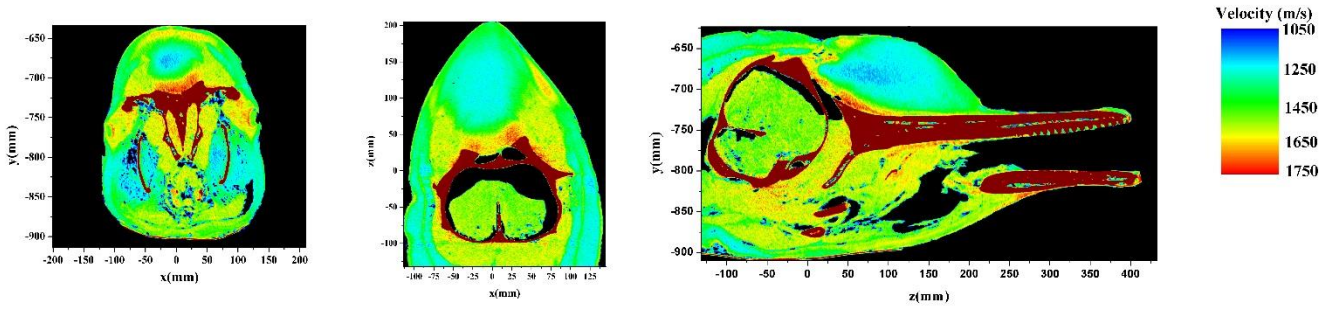

Fig. 6 (Color online) The estimated sound velocity distribution of an Indo-Pacific humpback dolphin (*Sousa chinensis)* head at 37°C, where the coronal cross section, the axis cross section, and the sagittal cross section are shown from left to right respectively. The icon on the upper right represents the value range of sound velocity.

Comparing the sound velocity distributions in Fig. 4b and Fig. 6, we demonstrated that the sound velocity in soft tissues decreased as temperature increased. The velocity change in soft tissues comparing 25°C to 37°C was estimated to 80-120 m/s for melon, and 10-15 m/s for muscle and connective tissue. These results were important for understanding how sound velocities were distributed in live tissues in

odontocetes.

IV. DISCUSSION AND CONCLUSIONS

Similar measurements have previously been presented from a juvenile Indo-Pacific humpback dolphin specimen.[25] However, the specimen was stored in formalin and then frozen for two years prior to analyses, causing the tissue atrophy and deterioration, which might make the sample data less reliable than that in the current paper. Though a previous study verified the reliability of the frozen and thawed postmortem dolphins to be representative of those *in vivo*.[29] No effect of chemical reagent, like formalin, on tissues deformation has been estimated. The atrophy in the forehead of the young Indo-Pacific humpback dolphin might be caused by the side effects of formalin.[25] The Indo-Pacific humpback dolphin here is a grown one in fresher state and kept in an ice chamber without any reagent influence and thus could be more representative of this species. Further, the density measurement was not tried in the previous work for this species.[25] The sound velocity and density measurements were both conducted here and could provide a more comprehensive understanding on acoustic properties of the Indo-Pacific humpback dolphin. Our results showed that for forehead tissues, melon had the lowest sound velocity and density. Muscle encased the melon with relatively higher sound velocity and density. The surrounding connective tissue had the highest sound velocity and density. This distribution of sound velocities followed the pattern found in other odontocetes,[26, 27, 30, 34, 35] but was different from the pattern presented in the younger Indo-Pacific humpback dolphin specimen by Wei *et al*.[25] Though the sound velocities of inner melon core and outer layer were given in the previous work.[25] The velocity distribution within the forehead was less reliable to show a same gradient trend as revealed here. In the velocity distribution given in the work,[25] the forehead soft tissue appeared to have two distinguishable components, connective tissue part and a part, in which melon was mixed with muscle. The tissue distribution was also different. In the sagittal direction, the connective tissue

layer located closest to the nasal passage and in front of connective tissue was melon layer. The muscle layer located at the front position of the forehead. The velocity of the soft tissue had gradients in front and rear directions. In comparison, the results in the present paper suggested the soft tissue was distributed with one layer encased by another and the velocity gradients distributed from inner melon core, muscle layer and outer connective tissue layer. The differences may either be age related reflecting different development stages of the tissues or related to the condition of the tissues as mentioned above.

However, there may also be other factors to explain the differences. Pressure and temperature have been shown to affect the sound velocity of the soft tissues in Cuvier's beaked whale, a dwarf sperm whale and a sperm whale.[26, 33, 36] These studies demonstrated that pressure changes could cause the sound velocities of the melon and spermaceti tissues to vary linearly. The relationships between the tissues' sound velocities and temperature were more complex, showing a positive linear relationship in Cuvier's beaked whale and negative nonlinear relationship in dwarf sperm whale and sperm whale. [26, 33, 36] Here, a trial was also made to investigate the effects of temperature on tissue sound velocity. In ocean sound communication, water acts as sound propagation medium, and changes of water temperature could bring massive effects on sound propagation. Similarly, the sound propagation and beam formation in odontocetes were related to the media, the forehead acoustic structures (skull structures, soft tissues and air components) and their respective properties. Therefore, if temperature causes velocity changes in soft tissues, logically the sound propagation and beam formation of the odontocetes could be influenced correspondingly. But this would need further studies on sound propagation and beam formation to verify. In the present paper, the reaction of soft tissue sound velocity to temperature was tested and the measurements suggested the sound velocities of melon, muscle and connective tissue pieces all had inverse relationships with temperature in the studied temperature range. Interestingly, among the soft tissues, melon had the highest velocity change, reaching

200 m/s within the temperature range, followed by muscle which had velocity change about160 m/s. The connective tissue had a velocity change of 70m/s for the temperature range. The velocity reactions of the melon, muscle and connective tissue to temperature were different and this might be caused by their composition differences,[32, 33] which also needed future studies to and was beyond the scope of present paper.

Here, temperature issue was addressed and our results showed a nonlinearity describing the tissues' velocity responses to temperature. In Fig.5, the rates (slope value) of sound velocity versus temperature in all three tissue types changed at a chosen temperature, 32°C. The altering point was chosen as 32 degrees to demonstrate the sound velocity rates of the relatively lower temperature range (20-32°C) and the high range (32-39°C) were different for melon, muscle and connective tissue. It could be clearly observed from Fig. 5 that the sound velocity of melon over 32°C was decreasing much faster than that below 32°C. This was also true from muscle and connective tissue. The altering temperature was chosen as 32°C to split the whole temperature range from 32°C to 39 °C into two ranges (20-32°C) and (32-39°C) to describe the velocity rate difference for these two ranges. But 32°C was not the only choice. Actually, the aim of choosing 32°C was to indicate and emphasize the velocity was decreasing at different rates in high and low temperature ranges, which corresponded to the nonlinear regression results. Other temperatures e.g. 30°C and 33°C might also be reliable. Here the temperature 32°C was chosen as a unified altering point for all soft tissues. The slope values shown in Fig. 5 clearly suggest the rates of the tissues change within the studied temperature range. The rate (slope value) below 32°C is -7.15 for melon and -17.05 above 32°C. For muscle and connective tissue, the rates below 32°C were -4.73 and -1.73 respectively, and those above 32°C were -13.85 and -6.77. Similar results have been reported in fats, the sound velocity of which decreases linearly as temperature increases until temperature reaches 35°C, at which point the fats begins to exhibit a nonlinear behavior in its sound velocity when temperature continues to increase over 35°C.[42]

Generally, the nonlinear effects of temperature on tissues sound velocities are common in studies on marine mammals and terrestrial mammals.[42] Studies have revealed that physical parameters, including temperature and pressure could influence the tissues' sound velocity. But how temperature and pressure changes the tissue sound velocity remains unknown. Previous research noted that the head tissues had different compositions[32, 47]. The fatty melon had lipid contents weighing over 90% of its total weight. For muscle components, lipid had a smaller weight proportion. As for lipid compositions, these tissues were also different, with fatty melon possessing more wax ester and less triglyceride than muscle. Temperature and pressure changes might change the internal molecular structures of the tissues' compositions and thus cause velocity differences at different temperatures. Soft tissues have been investigated to influence sound propagation and beam formation in odontocetes[37-40] and if its sound velocity was changed by temperature and pressure, the echolocation processed might be influenced as well. Within the dolphin head, the emitted sounds from the source propagated through its forehead acoustic structures, which contains soft tissues, skull structures and air components. These structures all play roles in beam formation, with skull structures reflecting the sounds and inducing interfacial waves, air components reflecting the sounds and soft tissues modulating the sound propagations.[37-40] The influence of temperature on soft tissues was investigated in this study. However, the influences brought by pressure as well as temperature on other acoustic structures, especially the fluid air components, were not researched and remained as future work.

The sound velocity data used in many dolphin simulation studies originate from measurements on postmortem samples under room temperatures e.g. 22.5°C.[26, 27, 30] Such results provide useful information to investigate how sounds are emitted and received in porpoises, dolphins and whales,[37-40] although they may be less representative of tissues *in vivo*. We estimated the sound velocity distribution at temperature 37°C on basis of the velocity distributions at temperature 25°C and the nonlinear relationships between

tissues' velocity and temperature. The method can be used to reconstruct the sound velocities under different temperatures. But here, sound velocity distributions of forehead tissues were estimated under temperature 37°C in that this temperature could represent the dolphin body temperature. The distributions could be referred to further studies on sound propagation and beam formation in dolphins *in vivo*. However, there are some limitations that need to be addressed in this approach too. We assumed that the sound velocity differences of melon, muscle and connective tissues between temperatures 25°C and 37°C applied to all available tissue pieces of melon (n=22), muscle (n=7) and connective tissue (n=13). The velocity differences were estimated from the three triple polynomial equations. It is possible that melon pieces in different locations may not follow the same value difference between the two temperatures, and this may also be true for muscle and connective tissue, however further studies are needed to verify this. In addition, a linear relationship between the samples' sound velocities and the HU values was obtained under the condition that the CT scanning results stay unchanged in temperatures from 25°C to 37°C. Generally, CT scanning experiments are conducted under room temperature, much lower than mammal body temperature. The scanning here was completed under room temperature 25°C after the specimen was thawed completely. But in Fig. 1, it could be clearly observed that the dolphin mouth is open. This was caused by its twisted tongue, shown in Fig. 1. As for the influences of temperature on CT scanning, to our knowledge, no trial has been made and future studies are required as well. Besides, the density of soft tissues under different temperatures were not tried here and also need subsequent studies. Regardless, the findings from our trials to reconstruct sound velocity in soft tissues of dolphins provide the first data set to investigate the sound beam formations in this species *in vivo*.

In conclusion, the reconstructions of the sound velocity, density and acoustic impedance distributions of an Indo-Pacific humpback dolphin head were completed here. The results show that like its odontocete

companions, the Indo-Pacific humpback dolphin also shows hierarchical properties in sound velocity, density and impedance distributions of soft tissues. For the soft tissues, nonlinear relationships between temperature and their sound velocities were revealed. The relationships were combined with the sound velocity measurement results of all 42 samples under temperature 25°C to estimate the samples' sound velocity at temperature 37°C. A new linear relationship between HU and sound velocities was extracted to reconstruct the head sound velocity distribution at temperature 37°C. A trial has been proceeded to a first reconstruction of an odontocetes head's sound velocity distribution at 37°C *in vivo*.The results presented here could provide important basic data to understand the sound propagation and reception process in this species.

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